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Experimental Model of Carcinoembryonic Antigen (CEA) Producing Human Hepatocarcinoma

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SUMMARY

The serially transplantable strain of the human hepatocarcinoma, with high production of CEA, was successfully established in nude mice. From this strain, a high CEA producing cell line was obtained.

Both the strain and cell line give us an ideal experimental model to study the mechanism of CEA production in hepatocarcinoma *in vivo* and *in vitro*.

Key words: Hepatocarcinoma, Experimental model, Carcinoembryonic antigen (CEA)

INTRODUCTION

The mecanism of production of alpha-fetoprotein (AFP) or of carcinoembryonic antigen (CEA) in hepatocarcinoma is one of utmost concerns in the fields of oncodevelopmental biology and medicine.

During the past decade, several investigators have been successful in transplanting human hepatocarcinoma into nude mice. No reports on the serially transplantable human hepatocarcinoma with persistant CEA production are known.

This paper aims to report on the establishment of a high CEA producing serially transplantable human hepatocarcinoma strain in nude mice and its cell line.

MATERIALS AND METHODS

1. Establishment of *in vivo* experimental model

Mice Four-week-old femal nude mice (BALB/c, nu/nu) were maintained in a vinyl isolator and kept under specific pathogen free (SPF) conditions.

Tumor transplantation The original tumor was obtained from a resected

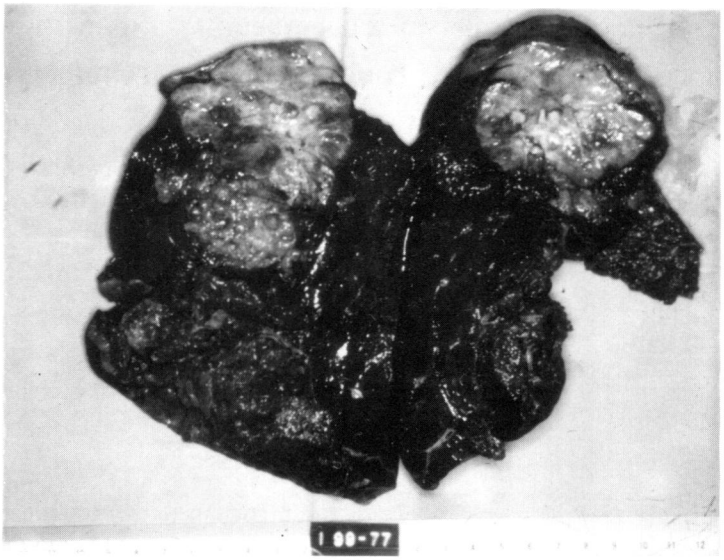

Fig. 1 Resected specimen of the original tumor

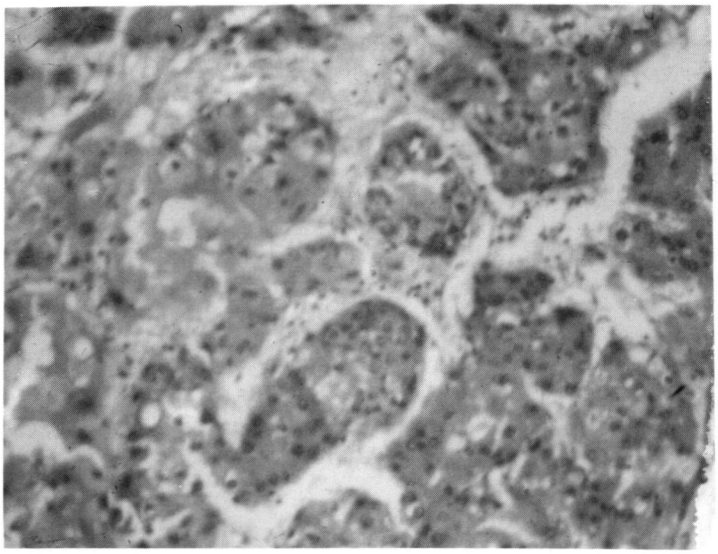

Fig. 2 Microscopic feature of the original tumor (H-E, ×200)

specimen of a 45-year-old male patient diagnosed to have hepatocarcinoma with cirrhosis, s/p extended right lobectomy (March 15, 1977) (Fig. 1). AFP and CEA values were 9,000 ng/m*l* and 2.5 ng/m*l* respectively. Histology of the liver tumor showed an alveolar pattern; the cells were varied in size and had hyperchromic nuclei (Fig. 2). The tumor tissue was diced into 2 mm cubes and inoculated subcutaneously at the back of the nude mice. Serial transplantation using subcutaneous graft of tumor fragment was done following the same procedure.

Examination of transplanted tumor Serial caliber measurements of three tumor dimensions were recorded twice a week after inoculation. The tumor volume was calculated based on the formula of Dethlefsen *et al.*(1) and the growth curve was drawn. Serum samples were collected and measured for AFP and CEA levels after each generation. The tumor tissues were processed for routine light, immunofluorescence microscopy and chromosomal analysis.

2. Establishment of *in vitro* experimental model

Cell culture On May 6, 1978, the tumor of 12 th generation was excised aseptically from nude mouse and was immediately treated for cell culture. The cell suspensions, after trypsinizing tumor tissue with 0.1% trypsin or 0.05% collagenase in calcium-and magnesium-free phosphate buffered saline (GIBCO) with 15% fetal calf serum and were incubated at 37℃ in 5% CO_2 in air in Falcon flasks. The medium was renewed twice a week and the subcultures were made by dispersing the cells with 0.04% ethylendiamine-tetraacetic acid (EDTA) 0.2% trypsin solution.

Examination of cultured cell Serial caliculation of cell number and inverted phase-contrast microscopic observation were done daily and the growth curue was drawn. The concentrated culture medium tested for the presence of CEA and tumor cells were then processed for immunofluorescence microscopy. Ten million tumor cells of the 20 th generation were transplanted into nude mice (back -transplantation) and the preveously mentioned examinations were done.

RESULTS

In vivo model Heterotrasplantation was successful in three out of four initial grafts. The incidence of takes in serial transplantation became 100% after the sixth generation. No metastatic lesions were noted. These tumors were designated as HC-4. The tumor growth became constant following the 11 th passage, began to grow 1.2 ± 0.4 days after transplantation (latent time) and reached a two fold increase in volume at a period of 5.1 ± 1.8 days (doubling time) (Fig. 3).

By radioimmunoassay methods, the human AFP and CEA concentration became constant following 23 rd generation and were measured at 4,040 ng/m*l* and 442 ng/m*l* in serum respectively at a tumor volume of about 3.0 cm^3 (Fig. 4).

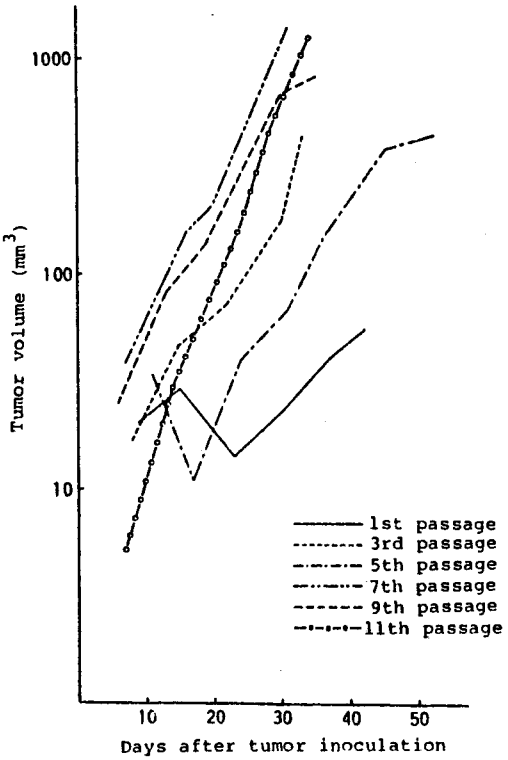

Fig. 3 Growth curve of HC-4

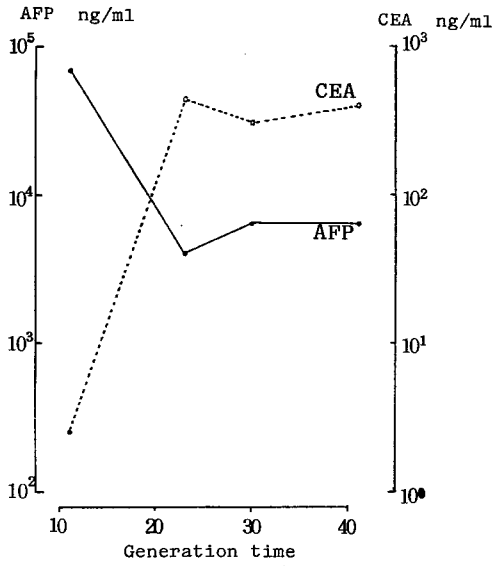

Fig. 4 Serum AFP and CEA levels of HC-4

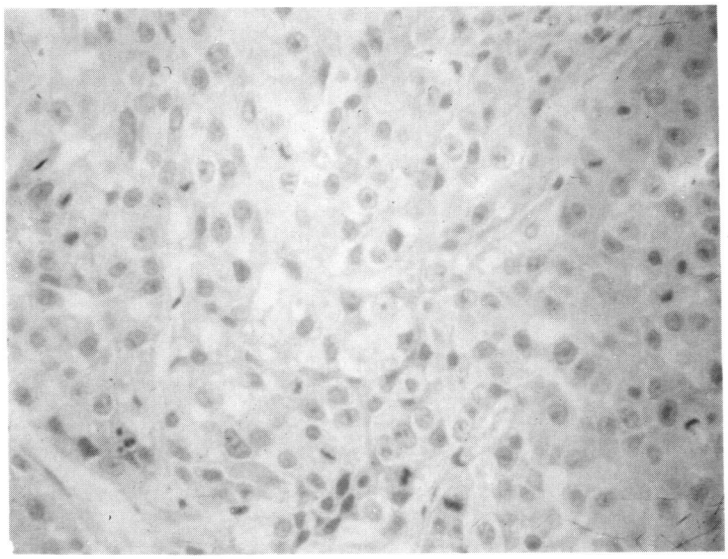

Fig. 5 Microscopic feature of HC-4 (H-E, ×200)

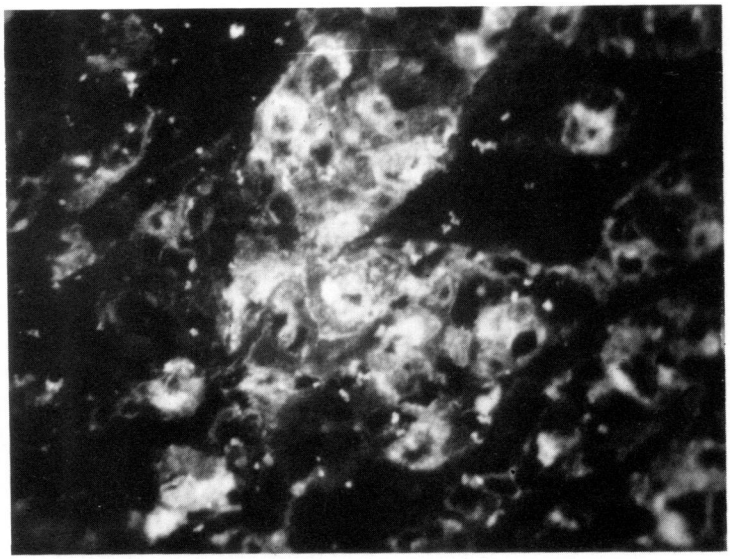

Fig. 6 Immunofluorescence stains of HC-4 (×200)

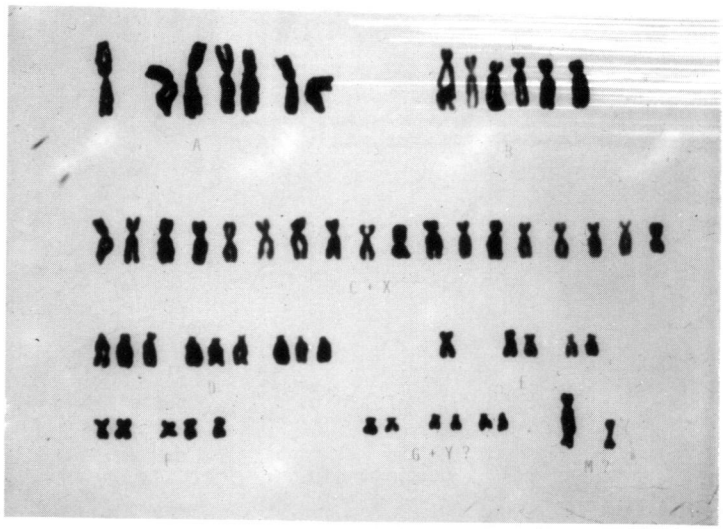

Fig. 7 Karyotype of HC-4

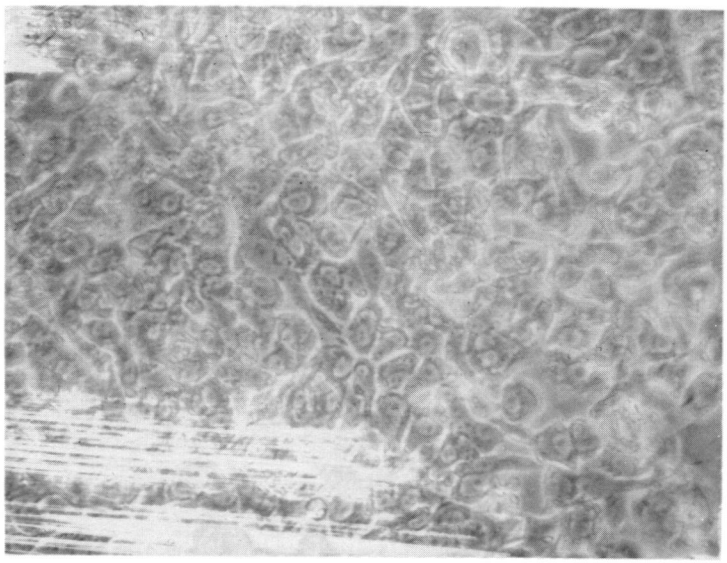

Fig. 8 Inverted phase-contrast microscopic feature of C-HC-4 (×200)

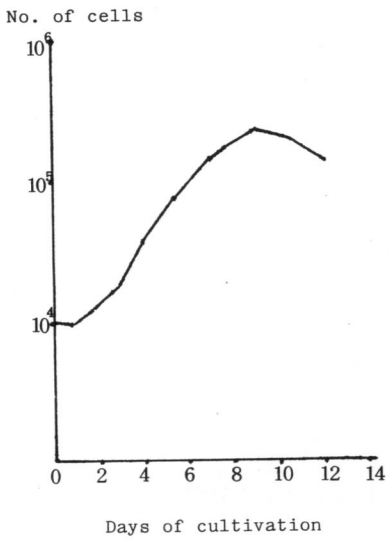

Fig. 9 Growth curve of C-HC-4

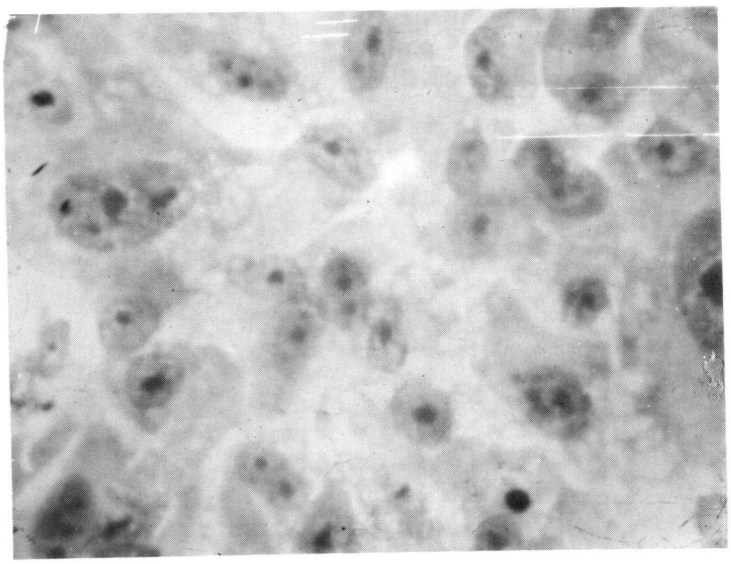

Fig. 10 Cytological feature of C-HC-4 (Papanicolau stain, ×400)

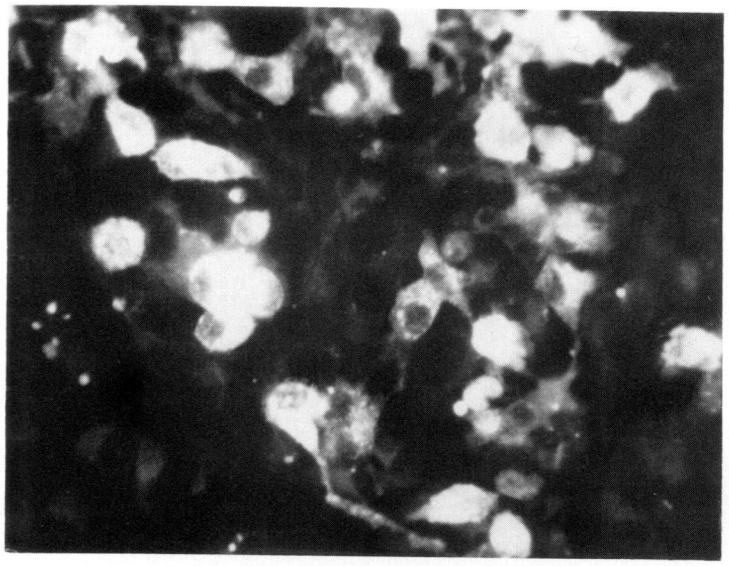

Fig. 11 Immunofluorescence stain of C-HC-4 (×200)

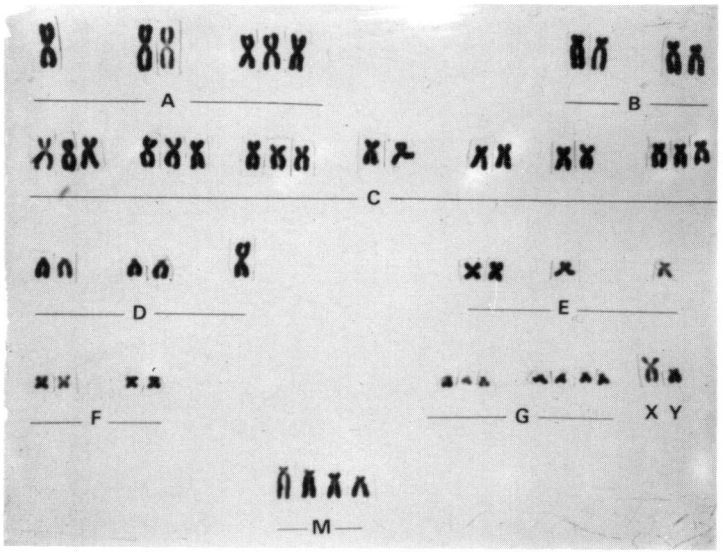

Fig. 12 Karyotype of C-HC-4

Grossly, the cut surfaces of the HC4 were greyish white and had occasional necrosis which was quite similar to that of the original tumor.

Histologically, the tumor was circumscribed with slight invasion to the overlying epidermis and adjacent adipose tissue. The alveolar pattern was not manifest due to a thin stroma. The tumor consisted of rounded or polygonal cells which were slightly larger than the original tumor and mitotic figures were much conspicuous. There was close structural similarity to the original tumor (Fig. 5).

On immunofluorescence examination, a intense membrane fluorescence of the fluorescein-conjugated anti-human AFP was observed in almost all of the HC4 cells (Fig. 6).

Chromosome study of the 12 th generation showed a human male chromosome constitution and a modal chromosome numbers is 58, most of which were metacentric and submetacentric (Fig. 7).

In vitro model At the time of primary culture, epithelial fibroblastic and hematopoietic cells were identified. Two weeks later hematopoietic cells began to disappear and 3 months, thereafter the cultured cells were composed entirely of typical epithelial cells with pavement-like arrangements. This culture of epithelial cells was named as C-HC-4 line (Fig. 8).

The cell growth of this line showed a lag phase in the first 24 hours and a exponential phase until the 5 th day. The doubling time was 20 hours (Fig. 9).

Cytologically, these cells were consistent of the polygonal cells with clear cytoplasm and marked mitosis (Fig. 10).

By the immunofluorescence technique, the intense membrane fluorescence of the human CEA was observed in all the C-HC-4 cells (Fig. 11). By method of radioimmunoassay, 20 ng/m*l* of AFP and 12 ng/m*l* of CEA per 5 m*l* of culture media without fetal calf serum were detected after 3 days cultivation of 10^6 cells in 110 th subcultivation.

Chromosome study in the 34 th subcultivation revealed a human male chromosome constitution and modal chromosome number of 52. Several marker chromosomes were found in addition to the gain or loss of A, C, D, E, and G group chromosome (Fig. 12).

The back-transplanted tumor reached 6.29 cm cubes tumor volumes after 3 months and had close histological similarity with the original human tumor. The human AFP and CEA concentrations were measured at 900 ng/m*l* and 23.9 ng/m*l* in serum respectively at this tumor volume.

DISCUSSION

The therapeutic result against hepatocarcinoma is still pessimistic and no other definite treatment has been found save for the radical resection of the tumor.

Therefore, the development of new treatment modalities poses a great challenge to all and at present there is no adequate experimental model of this tumor.

The mechanism of CEA production in hepatocarcinoma is one of the most interesting subjects in the field of oncodevelopmental biology and medicine today.

Since the report of Povlsen and Rygaard(2), several investigators have tried human cancer transplantation in nude mice. However, we are aware of only a small number of reports on the direct and serial transplantation of human hepatocarcinoma in nude mice(3). There are also few report on the CEA producing cell lines from human cancer(4, 5) but a CEA producing hepatocarcinoma cell line has not yet been reported.

We now report a successfully established high CEA producing serially transplantable hepatocarcinoma strain in nude mice and its cell line.

Histologically, the transplanted tumor (HC-4) was almost similar to that of the patient's. This strain (HC-4) was identified as human in origin by chromosomal characteristics as well as by the high production of human AFP and CEA. This strain retained its oncological nature as hepatocarcinoma throughout the 45 th generation for 5 years.

Serum examinations after the 11 th generation showed marked decrease of AFP and marked increase of CEA. This phenomenon is difficult to explain, however, we consider the production of CEA to be related to tumor proliferation based on the constant growth curve following the 12 th generation.

The establishment of cell lines of human hepatocarcinoma or hepatoblastoma with persistent AFP production has been said to be difficult because the cultivate cells often lose their capacity to produce AFP(6, 7). The reason for this loss of AFP production is long-term cell cultivation remains unclear and hypotheses have been suggested: (a) a mutation occurred *in vitro,* (b) synthesis of AFP was repressed by unknown factors in the course of cell culture and/or (c) overgrowth of non-AFP producting cells over the AFP producing cells.

Lastly, by immunofluorescence examination of the C-HC-4 cell line, a membrane fluorescence of human CEA was more or less observed in all cells. This may mean that cells simultaneously producing AFP and CEA exist.

In conclusion, our strain and cell line give us an ideal model to study the mechanism of CEA production in hepatocarcinoma *in vitro and in vivo.*

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